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(54) Analysis of biological products

(57) A process for the automatic and continuous identification, sorting and counting of small size particles, particularly biological materials, comprises preparing and depositing in a continuous process a succession of samples on a continuous mobile rest, spreading them out in the viewing path of a microscope, recording images of the samples produced by the microscope using a video system, dividing the image of each sample into a rectangular array of pixels, analysing the afore-said image according to pattern recognition techniques, and treating the results of the analysis statistically, dititizing and delivering them to an operator.

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SPECIFICATION

Analysis of biological products

The present invention relates to the analysis of biological products. More particularly the present invention is concerned with a new technique for automatically identifying, sorting out and counting particles of very small dimensions, particularly but not exclusively microorganisms of animal and plant origin, and also applying the obtained results to a variety of fields such as biological analysis (for instance of milk or blood), automatic controls for bacterial pollution (for instance in the automatic piloting of purifying stations for industrial effluents or drinking water). This new technique is characterized by its accuracy, speed and low cost.

Various processes aimed at achieving similar results are already known. For example in the case of human blood analysis, the usual technique consists first of all in preparing each sample manually by various mechanical and/or chemical treatments, then examining it under the microscope in order to analyse and count the different cellular elements under research. Such a technique is long and fastidious, while at the same time presenting a recognised measurement of error between 20 and 30%.

In the same way, in order to analyse and count the bacteria present in a liquid, the operator must first take samples of the liquid, then apply each one to cultures specially designed to show beacteria, which operation slows down the process by several hours, and eventually analyse and count the bacteria by reference to known forms and shapes, with the same limitations and inconveniences as described above.

More generally, there is at present no automatic and repetitive process capable of producing the desired results in a fast and continuous way, while at the same time avoiding human error.

The present invention aims to achieve this result through a succession of operations which are combined and adapted together in view of this new result.

The present invention provides a technique for automatic and continuous identifying, sorting out and counting of small size particles, characterized by the fact that it consists, in a first operation, of preparing and depositing in a continuous process a succession of samples on a continuous mobile rest, spreading them out on the exploration path of a video system recording the images of these samples provided by a microscope, then in a second operation implementing the afore-said recording, then in a third operation decomposing the image of each sample according to a squared lay out on view, and then in a fourth operation analyzing the afore-said image according to the "pattern recognition" techniques, and lastly treating the results of this analysis statistically, digitalizing and delivering them concretely to the operator.

The first operation consists of preparing and laying out, in an automatic and continuous process, a succession of samples on a continuous bed, and sorting them out in the viewing path of a video system recording each sample successively.

The second operation consists of the aforementioned recording with the video camera an image provided via a microscope, containing particular optical elements.

The third operation consists of dividing up the image of each sample according to a squared lay out featuring a number of lines and columns, and defining for each sample a certain amount of clearly identified zones or "pixels", in order to analyse each sample according to conventional techniques.

The fourth operation consists of the aforementioned image analysis according to the level of illumination of each pixel, by means of a computer, whose results for each sample are treated statistically then digitized on a printer, thus enabling one not only to know the result of the analysis at any given time, but also to follow in a real-time mode the evolution of the result.

Processing these various operations calls for a number of observations.

Observations on the first operation:

Each sample undergoes a physico-chemical preparation, previous to the stage of depositing on the rest or bed. This preparation is comparable to the usual treatment for the particular sample, but with certain modifications.

For example in previous techniques a "colouring" phase was only aimed at revealing a whole category of cells such as bacteria, without reference to their state of growth or death. Under the present invention however, colouring is used with the aim of indicating the viable condition of certain cells, and of visualizing them without colouring the artefacts.

In the same way, after colouring one can apply an epifluorescent treatment (generally UV rays from above) whose re-emission is filtered, which makes it possible to single out all cells containing a nucleus, and to extract them specifically by means of an immunofluorescent treatment.

Finally, each sample is automatically deposited on the continuous rest by pulverization or blowing, in the form of calibrated drops of constant thickness and diameter. These drops are spread out regularly and homogeneously on the rest, to be viewed successively by the camera. In a first development, the rest consists of a horizontal disc revolving around its axle, and the drops are deposited in concentric circles, so as to be explored either along

these successive circles, or by an alternative radial and circular exploration. In a second development, the rest is composed of a continuous tape unwinding from a feeding coil and winding up onto a receiving coil, the whole unit being housed inside a disposable cassette. The latter solution does away with the necessity of cleaning the rest as in the case of the disc. In general, 1 mm 3 represents the number of drops whose statistical result is chosen as sample value, and so forth for the following. Naturally, after each measurement the rest must undergo a cleaning operation, automatically controlled.

Observations on the second operation:

The system combines the use of a microscope fitted with special optics, and a video camera whose photo or image is transmitted to a mini-computer for the third operation. As the microscope transmits information continuously, it proves necessary to ensure that it is properly focused at all times. To that effect, a micrometer screw is placed between the optics and the sample rest, providing for constant mechanical focusing. The microscope will preferably be lit by direct UV, since the observations are being photographed by a camera, which can receive more light than the human eye.

Observations on the third operation:

The photograph of a sample is transmitted to the computer which then analyses it according to complex but well known techniques. Therefore it is not necessary to elaborate upon the description of these techniques. Suffice it to recall that the image being sent into the computer's memory ("acquisition"), one must first determine the minimum value of illumination of a pixel corresponding to a particle to be considered ("thresholding") then, once this threshold has been established for a sample, one can determine the contour of each particle. It will be noted however that it is particularly by this technique that the present invention differs fundamentally from conventional manual techniques, which limit themselves to a visual comparison with a series of given shapes. This difference shows both in the accuracy and in the speed of operation, as opposed to the simple visual comparison, which is slow, inaccurate, and can cause errors.

The following phase of this operation is the sorting out of the thus defined particles, according to their dimensions and shapes. Such a sorting out being implemented for each drop, the results of each drop are then treated statistically, which provides great accuracy in the information received, at any given moment and throughout their evolution in time.

Naturally, the general technique of the image or shape analysis ("pattern recognition") having become quite conventional, all other subsidiary operations known in this technique can be used, such as the pre-treatment of the image to eliminate "background noises" in order to obtain a clearer image, or such as filtering, dilatation, erosion or "skeletonizing" of the image, all of these techniques can be applied in specific cases.

The invention will be further described by way of example.

Example 1.

In this example, the technique of the invention has been applied to searching for bacteria in milk.

In the present case, the first operation consists of colouring the sample by means of orange acridine at pH 6. One can then observe in the drop: the fat globules, non coloured, the living leucocytes, with a shape similar to that of the fat globules but differing from them by their colouring, the bacilli, shaped like sticks, and the round shaped sheels, appearing with or without ribonucleic acid (RNA) in the process of "dying" and desoxyribonucleic acid (DNA), revivable. Thus the total number of germs and the number of leucocytes are known, which can be identified by epifluorescence, by illuminating from above at UV<400μ, after re-emission of a spectrum around 600 nanoμ which can be filtered.

One can then trace the clostridium butyricum and the pseudomonas fluorescens in the bacilli. Both can be extracted by the use of an immunofluorescence with a serum corresponding to the germ under research and a specific colouring. The following operations can then be implemented on the sample, always at low temperature:

a) One can obtain the total germs by adding 1% of cyanide and 10% of orange acridine to the sample, and by submitting some drops of the reaction product to operations 2 and 3.

b) One can obtain pseudomonas alone by repeating operation a), but without colouring.

c) One can obtain clostridium alone by repeating operation a), but adding both orange acridine and the specific coloured serum.

Whatever the a), b) or c) process chosen, the image produced by a microscope is photographed by means of a camera, the photo being transmitted through a computer's memory, in the form of a rectangle divided into 512 lines and 512 columns, which represents 262, 144 points or pixels. The depth of each point, that is to say the amount of light received, is represented by a figure or "bit". In the present case, six bits are used, which represents 64 levels, from zero as black to 63 as white. The images, being illuminated objects, stand out on a black background (so also do parasites). When starting an analysis operation, the minimum value of illumination (threshold) above which a point will be counted up as an object element is determined. Once this "thresholding" has been

established, the "contour" phase is started, that is to say the outline of each object is determined, then each object is itemized on a chart, according to the coordinates of each outline, from which a certain number of parameters can be established, such as "the greatest length", and also a "shape factor" which, starting from a circle arbitrarily representing 1, makes it possible to separate the "long" from the "round" objects. In the chart, objects corresponding to the chosen parameters are then singled out, and the results are eventually treated statistically, for each drop and for the whole of the drops. In the present case, the analysis of one drop represents one and a half seconds, with a recognized percentage of error of 5% can be achieved. These figures must be considered in relation to other data, such as the duration of each stage, and the margin of error presently admitted, as they result from the complete automation of operations.

Example 2: Blood analysis

In the case of blood analysis, the technique of the present invention not only provides great speed and accuracy in evaluating the cells themselves, that is to say red and white corpuscles, but also one can detect and count automatically parasites contained therein, which could not be done in this way before because of their very small size. For instance, it is now possible, thanks to the present invention, to research and count automatically the parasites of paludism, whose size is around 2 microns.

More precisely, blood analysis according to the technique of this invention is achieved through the following process:

First of all, the deposit on the rest is made in a continuous and automatic way by pulverization or blowing. This marks a definite progress compared to previous techniques, where each drop of blood was squashed between a rest and a glass plate, which would destroy or alter the cells.

The colouring phase with pH 6 orange acridine results in differentiation between the red corpuscles which, being without nucleus, contain neither RNA nor DNA and remain non-coloured, and the white corpuscles, being alive, containing a nucleus and various inclusions, which take up various colourings (DNA for the nucleus and RNA for the inclusions).

On the other hand, in the case of blood parasites being present inside a red corpuscle, such parasites will be revealed by colouring, as they contain DNA and RNA.

The image is analysed according to the general techniques previously mentioned, but taking into account the complexity of the "objects".

Indeed, if the white corpuscles, being coloured and well known, can be easily identified and counted by their contour, it is also possible to detect, within this outside contour, one or several inside contours which form nuclei, and inside which yet other constituents can be found. One can then, depending on the number of objects thus detected, identify and count different types of polynuclear constituents.

The same applies for the parasites of the red corpuscles, which is one important innovation of the present technique. For example, the parasite of paludism can be seen in the shape of an open ring (RNA) with excrescences or catkins (DNA) at both extremities, and this, in spite of their very small size (2 microns), they can for the first time, be counted up automatically and continuously in the direction of the analysis.

The particular application of this new technique, which was just mentioned at the beginning of this study, are in fact practically unlimited, extending in particular far beyond the mere counting up of particles in a liquid. One can likewise envisage applying it to the sorting out and counting of surface defects on solid materials.

CLAIMS

1. A process for the automatic and continuous identification, sorting and counting of small size particles, comprising in a first step of preparing and depositing in a continuous process a succession of samples on a continuous mobile rest, spreading them out in the viewing path of a video system for recording images of the samples by a microscope, a second step of implementing the afore-said recording, a third step of dividing the image of each sample into sections such as a squared lay out in view, a fourth step of analysing the afore-said image according to pattern recognition techniques, and a fifth step of treating the results of the analysis statistically, digitizing and delivering them to an operator.

2. A process according to claim 1, wherein the preparation in the first step comprises a specific colouring step, followed by observation by epifluorescence.

3. A process according to claim 1 or 2, wherein the deposit of the sample on the rest is achieved by continuous pulverization or blowing.

4. A process according to any one of claims 1 to 3, wherein the continuous rest consists of a glass disc revolving around a vertical axis, and undergoing a continuous cleaning process.

5. A process according to any one of claims 1 to 3, wherein the rest is a continuous tape winding from a first coil and onto a second coil during use, the tape being housed in a disposable cassette.

6. A process according to any one of claims 1 to 5, wherein the microscope is equipped with fittings to ensure permanent

automatic focusing on the sample.

7. A process according to any one of claims 1 to 6, wherein the sample is illuminated with UV light.

8. A process according to any of the preceding claims, wherein the colouring used is orange acridine and/or a specific coloured serum.

9. A process according to any one of claims 1 to 8 when applied to the determination of bacteria in milk.

10. A process according to any one of claims 1 to 8 applied to blood analysis, and more particularly to research of parasites such as the parasite of paludism in blood.

11. A process for the identification, sorting and counting of small particles, substantially as hereinbefore described with reference to the examples.

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